Genetic Models of Absence Epilepsy: New Concepts and Insights[☆]

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Introduction

Absence epilepsy was previously studied in chemical seizure models, in which absence seizures were pharmacologically induced either by systemic or local administration of drugs (such as a low doses of pentylenetetrazole in rats or mice, or penicillin in cats) and in single gene mutated mice. Pressure from society to abandon neurophysiological studies in cats, and the discovery that many (mainly albino) rats show spontaneously-occurring seizures, led to the development of genetic absence rodent models, such as the Genetic Absence Epileptic Rat from Strasbourg (GAERS) and the Wistar Albino Glaxo rats from Rijswijk (WAG/Rij). All individuals of these strains show an age-dependent increase in the expression of spike-and-wave discharges (SWDs) in their electroencephalogram (EEG), the hallmark of absence seizures. SWDs seen in these rodent models are similar to SWDs seen in absence epilepsy in humans, although clear differences in the frequency are dominant. In both species SWDs are accompanied by mild clinical symptoms including reduces responsiveness, in rats accelerated breathing, movements of the vibrissae, incidental twitches of fine facial muscles, and eye blinks can be seen in otherwise motionless animals. The interictal EEG of the rats appears to be normal. The SWDs appear suddenly on a normal background EEG and are bilaterally symmetrical, which is consistent with the classical view that absence seizures are generalized over the entire cortex of the brain, although SWDs are not well expressed at the occipital cortex. The topographical distribution of spike and wave differs: the spike is more clearly expressed in the frontal part of the cortex, while the wave is more pronounced in the occipital and parietal cortices. Differences in the topography of the spike and wave have also been described in human patients. Detailed neurophysiological and fMRI studies carried out at the beginning of this century were instrumental to new concepts on the origin of the SWDs.

Background

Many studies were designed in the eighties and early nineties of the last century to validate these genetic rat models as models of absence epilepsy. Pharmacological or predictive validity (how well outcomes of drug studies in the model predict the action of drugs in patients taking into account both false positives and false negatives) was investigated by testing antiepileptic drugs (AEDs), known to be effective or ineffective against absence seizures in humans. These studies showed that specific antiabsence and broad spectrum AEDs reduce SWDs, while drugs known to aggravate absence seizures in humans such as Na^+ channel blockers and GABA-mimetics do the same in these rodent models. Considering these outcomes, pharmaceutical companies now use WAG/Rij and GAERS to predict whether new compounds are effective in absence epilepsy or may aggravate absence seizures. Face validity was confirmed by the similar morphology of SWDs (clear negatively directed spikes followed by negative waves), by similarities in topographical distribution of SWDs, and by the frequency dynamics of long- and short-lasting SWDS in humans and rats. Other similarities contributing to face validity include the occurrence of SWDs in relation to the state of vigilance, a comparable circadian distribution of SWDs across the 24 h day, the provocative effects of sleep deprivation, and the corresponding clinical manifestations. Finally, the animal models have also construct validity since it was established that humans and rats have an

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identical behavioral (reduced responsiveness during SWDs is the key symptom of generalized epilepsies) and concomitant electrophysiological correlate of absences. The state of the brain during SWDs has been determined with evoked potentials; they are excellent indicators since the amplitude and latency of various components of these potentials are modulated by sleep and wake. It was found that auditory and visual evoked potentials, recorded in WAG/Rij rats, during SWDs most differ from those made during wakefulness and REM sleep and mimic those made during slow wave sleep while those during SWDs showed more after discharges. The latter suggested that the state of the brain and electrophysiological responsiveness during SWDs is similar to that during slow wave sleep with more network synchronicity. Also the reduced responsiveness, as determined with EEG desynchronizing responses to loud stimuli; was found to be comparable with slow wave sleep and SWDs. Also a comparison of the errors made in a time estimation positive rewarded task between WAG/Rij rats and children with absence seizures, showed that both species underestimate the passage of time when absence seizures occur. These results demonstrate that the decrease in the level of consciousness is a fundamental characteristic of absence seizures in both absence epileptic children and in the genetic rat models. It needs to be added that rats are not completely deaf and blind during SWDs, considering that the recognition of a relevant stimulus presented during an SWD is still intact, suggesting a reduced responsiveness, and not a complete lack of consciousness.

Initially the penicillin feline model, the local application of bicuculline on the cortex and thalamic slices of the ferret have been used to examine fundamental neuronal mechanisms of thalamic and thalamo-cortical interactions during SWDs; only later neurophysiologic studies were done in the genetic rodent models. In absence models a few important neurophysiologic multiple and single cell and intracellular recordings were carried out in vivo. The neuroanatomical pathways of the circuitry are well known. Thalamo-cortical and cortico-thalamic cells (including their long range cortical projections)—both with collateral afferents to the reticular thalamic nucleus (RTN), and with inhibitory projections from the RTN to the thalamo-cortical cells—are the primary pathways involved in the generation and propagation of seizure activity. A newly proposed essential part of the network are cortical columns in the somatosensory network. Early neurophysiologic studies indicated a critical role of the thalamus (including the RTN), since local field potentials showing SWD-like patterns were found there. More convincing evidence for the role of the thalamus is that in many thalamic structures, multiple unit activity is synchronized with the spike of the SWD (as recorded with cortical local field potentials). Whether the thalamus, and in particular the RTN, is also the initiator of the rhythmic oscillations (as has been proposed by the cortico-reticular theory, penicillin feline model) is less clear since particularly this is challenged in the rodent models. Some critical experiments to establish this, such as surgical isolation of the RTN, have not yet been done in genetic models and chemical and electrolytic lesions are difficult to carry out and interpret, considering the size and shape of the RTN. Therefore it remained therefore often uncertain, e.g., that intra-RTN injections of drugs are restricted to the RTN. This experimental point is important considering that the RTN contains only GABAergic cells and that the thalamus contains mainly glutamatergic ones. Therefore injections and lesions of the lateral thalamus are not always restricted to RTN. One outcome in favor of the important role of the RTN (at least in rodent models) in absence seizures is the upregulation of the T-type Ca^{2+} channels in the RTN in GAERS, the increased density of the $alpha_1$ 2.1 Ca^{2+} subunit current in the RTN in WAG/Rij rats, and the complete lack of the $\alpha 3$ GABA subunit immuno-reactivity at the inhibitory synapses of the RTN also in WAG/Rij rats. The lack of immuno-reactivity for a GABA subunit is particularly interesting, considering that deletion of the $\beta 3$ subunit in the RTN of knockout mice renders that part of the thalamus defective, showing a reduced intra inhibitory efficacy of RTN and enhanced tendency to oscillate. Further, recurrent inhibitory connections act as a desynchonizer of oscillatory activity in a healthy RTN, but this function is much reduced in mice and rats with a defective RTN.

Methods

Absences cannot be reliable established from clinical signs only. Therefore it is always imperative to record the cortical EEG in preferentially freely moving animals in order to quantify the number of seizures and to evaluate the effect of various experimental manipulations, such as systemic or local administration of drugs. The implantation of chronic epidural or subcortical electrodes for EEG recording or for electrical brain stimulation, and of cannulas for intracerebral injections of drugs, is a standard technique. Amplifiers with a high common mode rejection ratio, differential EEG recording, and impedance modifiers placed as close to the animals head as possible, contributes to the recording of a clean, artifact-free EEG in freely moving animals.

Methodological caveat: Outcomes of strain comparative studies (epileptic vs. nonepileptic controls) are difficult to interpret since a found difference cannot directly be ascribed only to the fact that one strain has epilepsy and the other not. Only when the epileptic rat is genetically identical to its control, and the control is without seizures, the interpretation of results from these studies is straightforward. In order to circumvent this interpretation problem, one can take advantage of the fact that young adult WAG/Rij rats (ca 2 months of age) do not have SWDs, while all 6 months rats show many SWDs. A difference in findings between 2 and 6 months old WAG/Rij rats can due either to the presence/absence of seizures, or to the age difference. In order to control for the age difference, one can include 2 and 6 months old rats of a nonepileptic strain—for example, ACI's which show no or very few SWDs at 2 and 6 months of age. Differences between younger and older WAG/Rij rats that are not present in the control strain can then be more readily ascribed to epileptogenesis or to the presence of seizures. This design has been often used. A solution regarding the most optimal control for GAERS are currently NEC (NonEpileptic Control). They were derived from the same Wistar colony and selected for being devoid of SWDs. Genetically speaking, they should resemble GAERS as much as possible, although through processes such as genetic drift it might be necessary to keep on characterizing them genotypically and phenotypically.

Recent Results

A Cortical Focus in Generalized Absence Epilepsy

The role of the cortex in the generation of SWDs has been investigated using cortical spreading depression techniques to transiently deactivate the entire cortex. During cortical deactivation, recordings have been made of the cortical and thalamic EEG in freely-moving GAERS and WAG/Rij rats. In GAERS, the corpus callosum has additionally been transected. At the height of spreading depression, when cortical inactivation is complete, SWDs are abolished in both cortex and thalamus. Spike-wave discharges reappear after a long recovery period, first in the cortex and later in the thalamus. Moreover, after callosectomy, the vast majority of SWDs are no longer bilaterally symmetrical, although occasionally bilateral synchronous SWDs are seen. The outcomes of this experiment demonstrates that a functionally-intact cortex is a prerequisite for spike-wave generation, that an intrathalamic circuitry alone is not sufficient to generate SWDs, and that each hemisphere contains an oscillatory system. Removal of the focal zone, previously only considered to be effective in partial epilepsies, has also been investigated. It was found that only bilateral cortical lesions of the somatosensory cortex abolished all SWD, although a lesion in the superficial layers is sufficient to reduce SWDs. This might imply that not only an intact cortico-thalamo-cortical network is imperative for SWD occurrence (the cortex and thalamus are mainly interconnected via cells in the subgranular layers), but that also intracortical pathways need to be intact for SWDs to occur. Neurophysiologic studies in GAERS extended the findings regarding the focal origin and identified highly excitable cells in the deep layers of the facial area of the somatosensory cortex.

Older studies in cortical slices of WAG/Rij rats showed both increased excitation and reduced inhibition in parts of the frontal cortex, and neurochemical studies show an expanded distribution of NMDA-mediated responses in the cortex of GAERS. These neurobiologic studies received new momentum after the discovery of a focal zone involved in the genesis of epileptiform activity in the cortex of WAG/Rij rats (Fig. 1).

Several laboratories have now confirmed the existence of a focal cortical initiation zone for SWD in WAG/Rij rats and in GAERS, but also in pharmacologic absence seizure models. Also pharmacological studies have shown that ethosuximide administered in the focal zone is more effective than injections in the RTN or the ventral basal complex of the thalamus. Injections of ethosuximide at other cortical locations are much less effective. Even phenytoin, a drug known to enhance SWDs when injected systemically, reduced SWDs after local injections in the cortical focal zone (Fig. 2). Other investigators have found a specific local increase in functional magnetic resonance imaging (fMRI) signals in the peri-oral somatosensory cortex during SWDs. These new discoveries have not only revived the classical debate about the origin of absence seizures. The identification of a specific cortical brain region from which the seizures are initiated has led to an attempt to determine why this zone is more excitable than other cortical zones. An upregulation in the expression of a subtype of Na^+ channels is specifically found in this region, and there are changes in I_h channels (which play a crucial role in the burst firing mode of thalamic and cortical neurons). Neurophysiologic in vivo studies in GAERS mainly done by the group of Stephan Charpier, specifically aimed at the cortical initiation zone, have shown that epileptic discharges are initiated in neurons of layers 5 and 6. These neurons, which show a distinctive hyperactivity associated with membrane depolarization, lead the firing of cortical cells during the epileptic discharge. Consistent with their ictogenic properties, neurons located in this focus exhibit interictal and preictal oscillations that are converted into an epileptic pattern. These pyramidal neurons of deep layers of the focal cortical site display an interictal subthreshold and suprathreshold activity that is markedly higher than that of neurons located in the upper layers. This sustained firing, composed of a mixture of single discharges and high-frequency intrinsic bursts, has a regular pattern. Consistently, short periods (0.2–1.2 s) of small-amplitude rhythmic membrane potential fluctuations, generating periodic firing during interictal epochs, are observed in these deep layer neurons. Throughout an SWD, the firing of layer 5 and 6 neurons systematically precedes the discharge of superficial neurons. Hyperactivity of deep layer neurons is specific to the focus, and the hyperactivity of deep layer neurons in the somatosensory cortex is specific for epileptic animals. Also a recent conclusion based on a extensive neurophysiologic study in GAERS was that SWDs in GAERS primary originate from aberrant activity of pyramidal neurons located in the deep layers of S1. The neuronal activity underlying these cortical SWDs in GAERS is investigated by concomitant recordings of surface EEG and intracellular activities of pyramidal neurons in layer V and VI of the facial region of the somatosensory cortex. Ictogenic neurons in this area show an excessive depolarized membrane potential with an increased probability of intense and regular spontaneous firing interictally. The occurrence of an SWDs in the EEG is accompanied by rhythmic neuronal depolarizations, which are superimposed on a tonic membrane hyperpolarization. The spike and wave components of the SWDs are accompanied by clear differences in membrane voltage and in probability of by depolarizing current injection induced action potentials.

A disregulation of inhibitory and excitatory synaptic systems may also participate in the generation of SWDs. The peak conductance of the fast component of inhibitory synaptic responses is reduced in deep layers of the focal cortical zone of WAG/Rij rats, documented in cortical-thalamic slices of WAG/Rij rats showing an increase in synaptic excitability mediated by NMDA receptors. Focal electrical stimulations elicit a late, large-amplitude, NMDA-dependent synaptic depolarization that can trigger repetitive firing. A similar stimulating protocol in control Wistar rats produces only a monophasic synaptic depolarization that can trigger only a single action potential. These recent neurophysiologic data obtained in GAERS and WAG/Rij rats provide a firm underpinning of the cortical focus theory of absence epilepsy. The excitatory cortico-thalamic inputs from cortical layers 5 and 6 (of the initiation zone) are stronger than in nonepileptic animals, and they impinge upon the thalamic relay and higher order cells and the RTN with its decreased propensity to dampen oscillations. These changes in intrinsic and synaptic properties facilitate resonances and spread throughout the thalamus, and propagation back to the cortex.

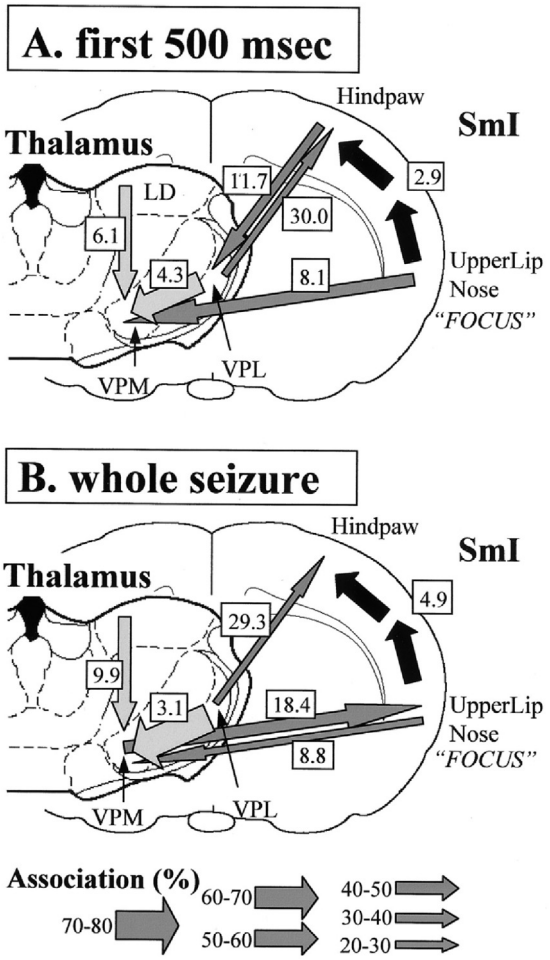

Figure 1 Summary of the corticocortical (represented by *black arrows*), intra-thalamic (*light gray arrows*), and corticothalamic (*dark gray arrows*) interdependencies during spontaneous absence seizures in the WAG/Rij rat as established by the nonlinear association analysis of a representative WAG/Rij rat (10 seizures were analyzed). The *thickness* of the *arrow* represents the average strength of the association, and the *direction* of the *arrowhead* points to the direction of the lagging site. The values represent the corresponding average time delays in milliseconds. *A*: The relationships as found for the first 500 ms of the generalized seizure showed a consistent cortical focus in the perioral somatosensory cortex (*SmI*), because this site consistently led the other cortical recording sites. The hind paw area, for instance, was found to lag by 2.9 ms on average with respect to this focal site. Within the thalamus, the laterodorsal (*LD*) nucleus was found to consistently lead other thalamic sites. The ventroposterior medial (*VPM*) nucleus was found to lag behind the ventroposterior lateral (*VPL*) nucleus, with an average time delay of 4.3 ms. Concerning corticothalamic interrelationships, the cortical focus site consistently led the thalamus (*VPM*), with an average time delay of 8.1 ms. Within the somatosensory system of the hind paw, the (nonfocal) cortical site led the thalamic site (*VPL*) during 3 of 10 seizures; the thalamus led the cortex during 1 seizure, whereas for the other 6 seizures no direction of the delay could be established. *B*, The relationships as found when the whole seizure is analyzed as one epoch. The same cortical focus as during the first 500 ms was found consistently. Compared with the first 500 ms, the time delay from the cortical focus with respect to the nonfocal cortical sites has increased. Furthermore, the strength of association between VPL and VPM has increased. The direction of the cortico-thalamic couplings has changed. For the nonfocal cortical sites, the thalamus was found to lead during all seizures. For the focal cortical site, the cortex was found to lead during two seizures, whereas the thalamus was found to lead during seven seizures. From Meeren, H.K., Pijn, J.P., van Luijtelaar, E.L., Coenen, A.M., Lopes da Silva, F.H., 2002. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J. Neurosci. 22, 1480–1495.

Considering that neurophysiologic studies can necessarily cover only a restricted part of the brain, it is imperative that that an initiating role of other parts of the brain can be excluded. Therefore brain imaging studies with a high spatial resolution such as fMRI are badly needed. Various MRI studies were conducted in WAG/Rij rats. In the first studies, the BOLD signal was coupled to the EEG in awake previously habituated to the experimental conditions or in fentanyl-haloperidol anesthetized rats. T2-weighted echo planar imaging at 4.7 and 7 T respectively showed an increase of more than 6% of the BOLD signal in the sensory, parietal and temporal cortices as well as in the reticular, mediodorsal, ventroposterior and posterior nuclei of the thalamus during SWDs versus interictal activity. No significant changes were seen in temporal or limbic structures (e.g., hippocampus) and no significant negative BOLD signal was observed for any seizures. These data do not confirm that the whole brain is involved, they are however in agreement with neurophysiologic data showing the role of the somatosensory cortex and some higher order and relay thalamic nuclei in

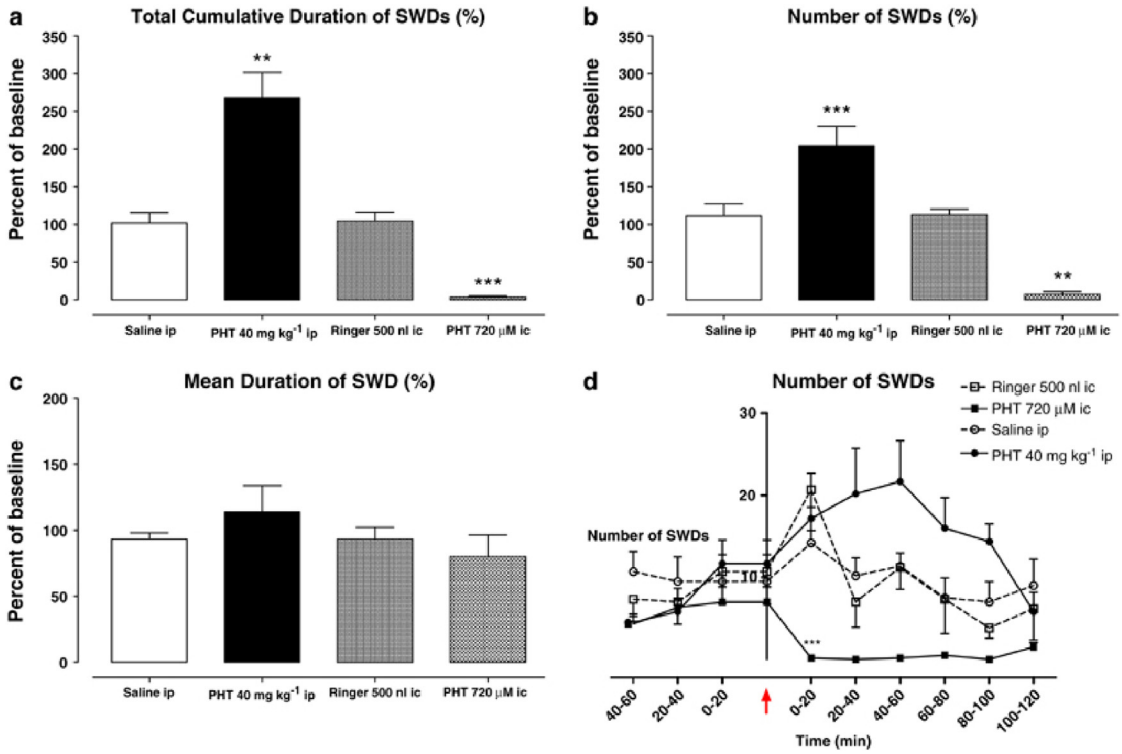

Figure 2 Effect of Phenytoin on total cumulative duration (A), number (B) and mean duration (C) of SWDs throughout 2 h post injection and the number of SWDs per 20 min intervals (D) before and after i.p. and intracortical (i.c.) injections in WAG/Rij rats in the deeper cortical cell layers ($n = 7$). $^{**}p < .01$; $^{***}p < 0.001$. From Gurbanova et al. 2006. Br. J. Pharmacol.

the generation of SWD (see below). When 7 T MRI was used, however, the increase of BOLD signal was also observed in the hippocampus, the basal ganglia nuclei, the tectum and the tegmental nuclei. The changes observed in the latter structures remain to be further examined but some of them appear in agreement with the involvement of basal ganglia structures in the control of SWD. fMRI combined with EEG in GAERS was also used to determine the directionality of interactions including establishing neuronal drivers between the epileptogenic zone in the somatosensory cortex and more remote brain regions. SWD-correlated bilateral changes in CBV were confirmed for S1 and thalamus, beyond that changes were found in the brainstem, cerebellum, SNR, striatum, and different cortices (retrosplenial, visual, limb region of S1, and motor and sensory secondary). The neural driver of SWDs discharges based on the intracranial EEG was indeed estimated to be at S1BF.

Diffusion tensor imaging was used to characterize long-range white matter network modifications. Presymptomatic and symptomatic WAG/Rij rats at two different developmental stages (1.7 and 8 months) were compared to age-matched nonepileptic (control) Wistar rats. Symptomatic WAG/Rij exhibited a localized decrease in FA (fractional anisotropy, a measure of structural integrity of white matter) in the anterior part of the corpus callosum, compared to both controls. Also GAERS exhibited a marked decrease in FA in the anterior corpus callosum vs. age-matched NEC. This decrease was more extensive than in WAG/Rij rats. Symptomatic WAG/Rij and GAERS also had an increased perpendicular diffusivity ($\lambda^{\perp}$) in the anterior corpus callosum, which could be the cause of the reduced FA observed in the epileptic animals. The white matter pathways of the anterior corpus callosum interconnected the fibers of the facial region of the somatosensory cortex between two hemispheres. This suggest that SWDs lead to microstructural changes in white matter pathways interconnecting the regions where the SWDs have their site of origin and that white matter abnormalities could contribute to chronic dysfunction in what has classically been considered a gray matter disorder.

Network Analyses and Circuitries

A further elaboration of the cortical focus theory requires a better understanding of the local and global mechanisms of absence epilepsy, as well as of the spread of epileptic activity over the cortex. We have hypothesized that the epileptic focus in the perioral region of the somatosensory cortex interacts with functionally and anatomically related regions—i.e., the adjacent parietal and frontal (sensory and motor) areas, creating an oscillatory loop that generates SWDs. When this occurs, then the onset of SWDs is associated with a significant increase of fronto-parietal coherence (and less significant changes of coherence between distant and functionally divergent areas, such as the fronto-occipital and parieto-occipital areas). Coherence, like correlation, is a measure of the interdependency of two signals; a high correlation suggests coupling of the signals. In our experiments, coherence was

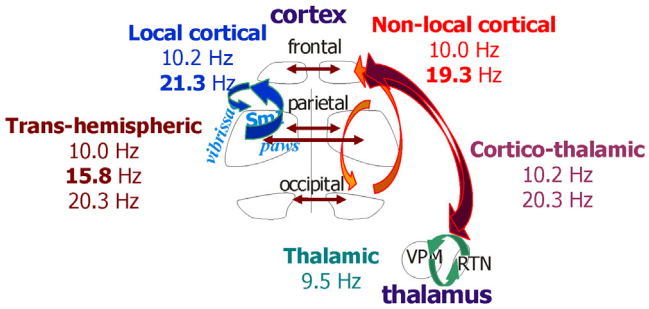

Figure 3 Outcomes of coherence analyses of local field potentials as produced in cortico-thalamo-cortical circuits. Five resonant circuits are identified during an SWD. The first circuit is at or near the site of origin, the parietal cortex. The second one is the trans-hemispheric network, it connects the two hemispheres; the third one is the nonlocal cortical network, it associates the parietal cortex with the occipital cortex; the fourth and fifth are the cortico-thalamic-cortical reverberating and local thalamic activity. The dominant oscillating frequency is around 10 Hz and its harmonic 20 Hz, the trans-hemispheric circuit has an additional 15 Hz oscillation. After Sitnikova, E., van Luijtelaar, G., 2006. Cortical and thalamic coherence during spike-wave seizures in WAG/Rij rats. Epilepsy Res. 71, 159–180.

calculated from local field potentials recorded from various epidural and thalamic electrodes, placed intra- and interhemispherically in WAG/Rij rats. Coherence coefficients showed that the onset of SWDs is associated with a strengthening of both unilateral and bilateral intracortical and unilateral thalamo-cortical interactions. A significant increase of cortico-cortical, cortico-thalamic, and thalamo-thalamic coherence is detected in frequencies from 5 to 60 Hz. The highest increase of coherence is obtained in two frequency bands—around a mean frequency of SWDs (8–11.5 Hz) and in the first harmonic band of 16–21.5 Hz. Moreover, the anterior cortical areas have an intrinsic propensity to hyper-synchronize, which facilitates unilateral and bilateral propagation of SWDs over anterior cortical areas. Nonsurprisingly, the trans-hemispheric cortical coherence is higher than the intrahemispheric cortico-cortical and cortico-thalamic and thalamo-thalamic coherence. This implies a large and crucial involvement of the corpus callosum in the pathophysiology of absence seizures and the interconnected fibers originating from the left and right focal zone. Strikingly, an SWD-related decrease of coherence is found in a narrow low-frequency band (<5 Hz), limited to cortico-cortical couples formed by the perioral region of the somatosensory cortex and the thalamo-thalamic pair. Since a similar pattern of coherence changes is seen in the cortical and thalamic local oscillatory networks, it appears that there is a common neurophysiologic mechanism controlling intracortical and intrathalamic synchrony. The onset of SWDs is associated with a higher functional coupling throughout cortico-cortical, thalamo-thalamic and cortico-thalamic associative pairs. This increase of oscillatory synchrony is topographically and frequency specific, suggesting that a functional relationship explicitly distinguishes different oscillatory circuits. This finding suggests that there is nonhomogeneous spread of SWD over thalamo-cortical system, and is inconsistent with the former viewpoint that SWDs occur without localizing features (Fig. 3).

Detailed studies of interrelationships between local field potentials, quantified by means of more advanced signal analysis methods of nonlinear association, yields a direct measure of the degree of correlation between the events recorded at different sites (i.e., it gives an indication of the degree of functional coupling between the underlying neuronal populations). In addition, the method provides a time delay between signals. Together, these two parameters deliver evidence about "driver-response" relationships between the respective neuronal populations. The outcomes of the analyses of the cortico-thalamo-cortical, intrathalamic, and cortico-thalamic network in WAG/Rij rats activities with a sliding or moving window covering EEG segments before, during and after the occurrence of SWDs, showed that SWDs (it is obvious that this discovery is in contrast to what has generally been assumed, although also earlier studies emphasized the leading role of the cortex) have a cortical focal zone of initiation in the peri-oral region of the somatosensory cortex. The activity quickly spreads from this zone to the more superficial zones, next over the cortex, to the contralateral hemisphere and to the ipsilateral thalamus. Therefore, it seems that the thalamus is only secondarily involved. Moreover, thalamocortical cells are only weakly active during SWDs in GAERS, in contrast to what was assumed based on early in vitro studies of thalamic burst firing patterns. However, the TC cells interconnected with the RTN do provide a resonant circuitry that sustains the cortical discharges.

Considering that the thalamus contains many different structural and functional parts (arousal, limbic, sensory and motor), the role of intrathalamic and cortico-thalamic networks with various state of the art signal analytical techniques, such as nonlinear association analyses, pairwise phase consistency analyses (PPC), a frequency resolved, nonparametric Granger Causality (GC) analysis and a nonlinear Granger method, has been investigated. These methods are used to study the dynamics of cortico-cortical, cortico-thalamic, thalamo-cortical and thalamo-thalamic network activities once more with a moving window. Here we focus on the main common findings; it is obvious that the different methods give sometimes different outcomes considering that some methods are linear, some not, one was amplitude based, another used phase, some were more robust than others and that different interictal control periods are used. PreSWD changes in network activity are first noticed in the deep layers of the somatosensory cortex, about 2 s before the onset of the generalized (first large spike of a train of spike and waves in cortex and thalamus) with the aid of the nonlinear GC (see Figs. 4A and 5). Next first unidirectional increase (feed forward steering) in coupling is noticed in a restricted number of cortico-thalamic pairs, among others with the strongly reciprocally connected posterior thalamic nucleus, later but still

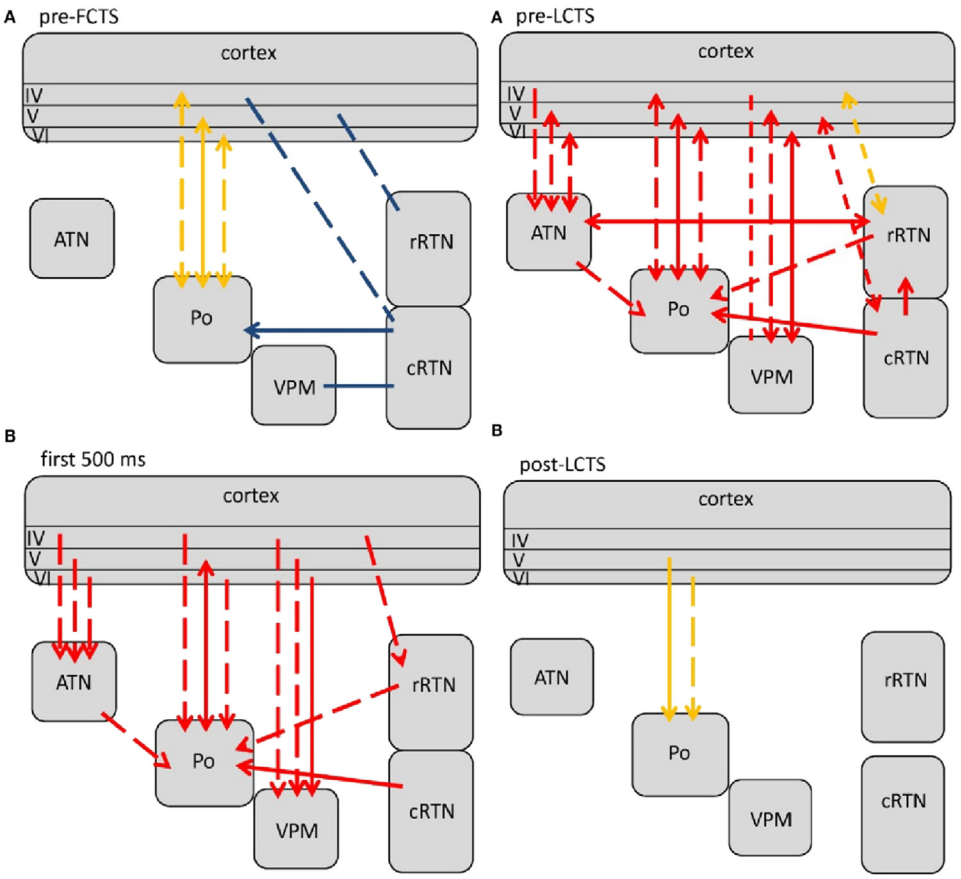

Figure 4 (A) Changes (as compared to nonepileptic control) in network interactions seen with SWD generation. All preictal changes are represented in *(A)*. Earliest preictal changes were detected up 1.25 s prior to SWD onset or better the FCTS (first cortico-thalamic spike of the SWD). *(B)* Represents network coupling seen within the first 500 ms following FCTS. *Solid lines* represent changes between anatomically connected structures, *dashed lines* changes between structures that do not possess a direct, anatomical connection. The direction of coupling is indicated by *arrowheads*, which can either be unidirectional (→) or bidirectional (↔). Orange indicates a significant increase that did not yet reach its maximal value at that time point, red indicates that the coupling reached its maximal value and blue indicates a decrease in coupling. (B) Changes in network interactions seen with SWD termination. *(A)* Represents changes seen from about 1.5 s prior to the LTCS (last cortico-thalamic spike of the SWD) until LCTS. *(B)* Represents changes from LCTS until about 1.5 s following it. *Solid lines* represent changes between anatomically connected structures, *dashed lines* changes between structures that do not possess a direct, anatomical connection. The direction of coupling is indicated by arrowheads, which can either be unidirectional (→) or bidirectional (↔). Orange indicates a significant increase that is not at its maximal value at that time point, red indicates that the coupling is at its maximal value (Lüttjohann and van Luijtelaar, 2015).

before SWD onset, in a bidirectional fashion (feed-back). Also other cortico-thalamic and thalamo-cortical channel pairs turned into bidirectional coupling approaching SWD onset. Also a gradual increase of intrathalamic coupling was detected with the nonlinear GC method, but interestingly, in some thalamic pairs a decrease in phase consistency was found immediately before SWD onset.

Although the cortex guided various thalamic nuclei in the first 500 ms of the SWD, the PO was the only nucleus which kept its bidirectional coupling with the PO. Both the rostral and caudal part of the RTN guided the PO. SWD onset is also characterized by a nonlinear coupling decrease for more than a second in a majority of channel pairs, only the cortex kept driving the cRTN. In the course of the SWD, more cortico-thalamic and thalamo-cortical channels showed an increase in bidirectional coupling which kept increasing toward the end of SWDs. The increase suddenly vanishes at SWD offset; this vanishment was preceded by an diminishment of the cortical drive to the rRTN and by an increase in the directional drive from the cRTN to the rRTN, providing a clue on how SWDs are aborted see Fig. 4, right top and bottom.

It can be concluded that network analyses clearly show early preictal changes in network activity in the deep layers of the somatosensory cortex in line with the cortical focus theory, a gradual involvement of different thalamic nuclei via feed-forward and later feedback control until SWD can be seen in the thalamus. Network analyses also shows that the thalamus is not homogenous regarding the role in generating SWD oscillations, in sustainment and in SWD offset. The rRTN might have a resonator function, not a pacemaker function as was previously proposed. It passively receives oscillations from the cortex, and might play a role in synchronized thalamic firing via its wide projections to both limbic parts and relay nuclei. The cRTN might inhibit SWDs through

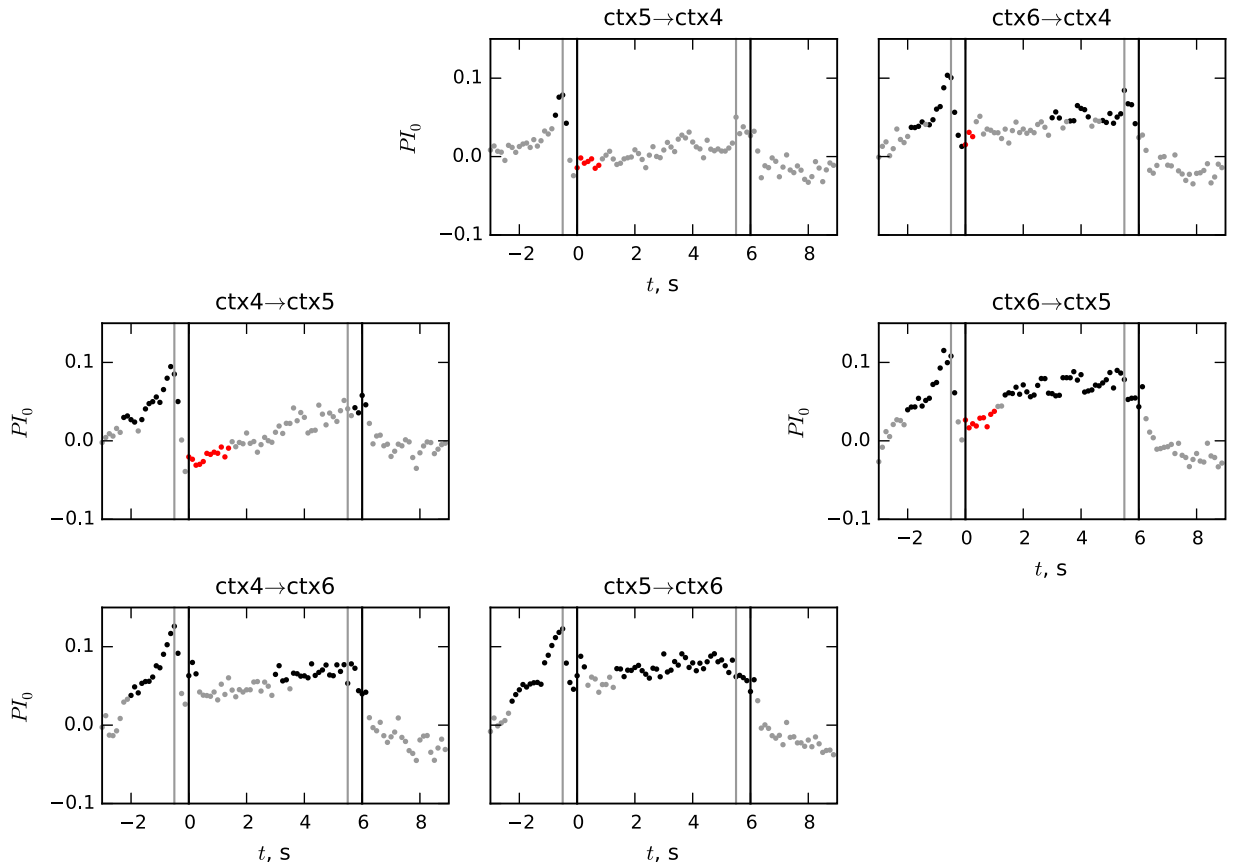

Figure 5 Dynamics of adapted nonlinear Granger causalities for intracortical channel pairs, electrodes were aimed at facial region of the somatosensory cortex layers IV,V,VI. Y-axis: Prediction Improvement (*PI*) normalized to baseline level (10–3 s before onset). *X*-axis: time, the moment of SWD onset is considered to be at $t = 0$. *PI* was averaged over 16 rats. *Black vertical lines* indicate the seizure onset and offset, *gray vertical lines* indicate the length of moving window, in which Granger analysis was performed. *Black points* indicate values significantly larger than zero (baseline *PI* from 10 to 3 s prior to SWD is treated as a zero level) based on Student *t*-test, *red points* indicate values significantly lower than the preictal maximum, gray points—all others. In all cases only if there are at least three significant values in a row, they are considered as truly significant in order to avoid false positive due to repetitive testing (Sysoeva et al., 2016). The figures indicate that more than 2 s prior to SWD onset, the cortical layers start to influence each other until SWD onset. Next, there is a sign decrease in coupling in most channelpairs, followed by a gradual increase in bidirectional coupling (feed-forward and feed-back steering) with cortical layer VI toward the end of an SWD (Sysoeva et al., 2016).

a increase in directional coupling with the rRTN, also since lesions of the doral lateral thalamus, including the cRTN, increased SWD. Although visual analyses may suggest that SWDs start and end quite abruptly from a normal appearing cortical desynchronized background EEG, network and precursor analyses show clearly otherwise. Different key players and networks seem responsible for initiation and abortion of SWDs.

Genotype-Environmental Interactions

Although generalized seizures (including absences) have a genetic origin, it can be clinically relevant to know how the genotype and the environment interact to modulate and shape the epileptic phenotype. To study this interaction, we subjected the WAG/Rij and a nonepileptic control inbred rat strain (ACI), which differ significantly in their genetic seizure susceptibility, to various environmental manipulations during different stages of development. We also exposed pregnant WAG/Rij mothers to PTZ, WAG/Rij pups were exposed to maternal deprivation and neonatal handling, weaned ACI and WAG/Rij rats were housed in either an enriched of impoverished condition in the period that SWDs start to emerge (0–60 days post weaning) and when they become fully mature (70–130 days post weaning). The EEGs of these ACI and WAG/Rij rats were recorded when they were adult. The outcomes of the pregnancy study show that repeated pentylenetetrazole (PTZ) injections during pregnancy protect WAG/Rij rats against SWDs and PTZ-induced seizures until about 70 days of age; older rats are more sensitive than younger WAG/Rij rats to a challenge with PTZ. Next, it was found that the occurrence of seizures was only minimally affected by housing conditions, suggesting that SWDs are mainly controlled by genetic factors. The mean duration of SWDs was affected though. It does show that different seizure characteristics such as number and mean duration have a different heritability and show a different sensitivity for the environment.

Manipulations during the weaning period are not only more successful in changing the epileptic phenotype than the same manipulations post weaning, the changes in seizure activity after early environmental manipulations (such as maternal deprivation and neonatal handling before weaning) are accompanied by an increase in the expression a subtype (HCN1) of I_h channels and a decrease in SWD. The changes in this HCN1 subunit, a subtype of I_h channels, precede the age-related development of SWDs, and the loss of the dendritic I_h might form the basis for the initiation of the spontaneous seizures in the focal zone. The early manipulations may affect seizure activity and I_h channels in the cortex are critically involved in the pathogenesis of absence epilepsy. All this suggests that interventions based on the prevention of or compensation for the loss of I_h channels might be a new therapy for absence epilepsy. Subchannel selective agents are not available which hampers progression.

Antiepileptogenesis

Neither WAG/Rij nor GAERS have SWD at weaning. At 3–4 or 5–6 months, all GAERS and WAG/Rij rats respectively have hundreds SWD per day. The age dependent increase in morphology, number and mean duration demonstrates that epileptogenesis is occurring. It needs to be emphasized that little is known about the underlying factors of epileptogenesis, only a few correlates of epileptogenesis have been identified in the focal zone, such as an age dependent increase in a subtype of Na^+ channel and a decrease in HCN1 channel, changes in mGlu1R and mGlu5R, diminishment of parvalbumin containing cells over widespread cortical zones (not exclusively in the focal region), reduced expression of $GABA_B$ receptors in the somatosensory cortex. Other age-dependent changes are the increased expression of mGlu2/3 receptors as assessed by immunohistochemistry and Western blotting in the somatosensory cortex. In contrast, mGlu2/3 receptor signaling as assessed by the ability of the agonist, LY379268, to inhibit forskolin-stimulated cAMP formation was reduced in slices prepared from the somatosensory cortex of 6-month-old WAG/Rij rats compared to "presymptomatic" 2-month-old WAG/Rij rats.

Antiepileptogenesis in the form of a firm reduction in SWDs after the treatment had stopped, including a reduction of the depression-like behavior but also in cortical excitability and white matter changes has been established in WAG/Rij rats, first by chronic ETX treatment, later also after early and chronic of vigabatrine and levetiracetam, making it less likely that only T-type channels are involved in this process. In GAERS, it has been established that antiepileptogenesis induced by early and chronic ETX treatment alters epigenetic processes. More specific the increased expression of increased DNA methyltransferase (DNMT, DNMT enzymes catalyze DNA methylation) as found in the focal area is considered as strong evidence for reduced epigenetic modifications.

Interaction Between Various Types of Epilepsy

The treatment of complicated mixed forms of epilepsy is one of the most challenging tasks confronting the clinical epileptologist. The suppression of one kind of paroxysms can be accompanied by the aggravation of other seizure types. Whether and how a seizure type develops in the presence of another type is completely unknown. We have taken two approaches to investigate whether there is an interaction between two different seizures types. In the first line of research, WAG/Rij and control rats were selected for their audiogenic sensitivity. Behavioral and neurochemical differences in some monoamines, as measured in extracted tissue immediately after exposure to the sound stressor, were investigated. The presence of absence seizures correlates with novelty-induced anxiety, in which the limbic circuitry seems to be involved. Sound stress activates the 5-HT system differently in the different subject groups: audiogenic-sensitive rats and rats with only absences display predominantly a reaction in the thalamus, whereas nonepileptic rats show the highest response in the frontal cortex. The highest thalamic response occurs in rats with absence epilepsy, with much smaller changes in audiogenic sensitive control rats. We conclude that the brain 5-HT-ergic response to stress depends on the type of epileptic pathology.

A second approach to the interaction between two seizure types has made use of the kindling model. Both GAERS and WAG/Rij rats show peculiarities in the kindling process when compared with control rats. While controls are fully kindled after 12 to 15 electrical stimulations in the amygdala, GAERS remained at the initial stage 2 of kindling even after 30 sessions, with no motor seizures observed. Moreover, the duration of the after-discharge after the kindling stimulus is shorter in GAERS. WAG/Rij rats are also more difficult to kindle; it takes more sessions before some of them reach the end stage 5 of kindling. Resistant rats have more SWDs preceding the kindling sessions. Thus, it appears that kindling of genetic absence epileptic rats is strongly suppressed—perhaps because of the daily occurrence of a large number of absence seizures of these rats. This results suggests that the limbic system is changed in these genetically absence rats—which is perhaps surprising considering that SWDs occur in the cortico-thalamo-cortical circuitry and that mechanisms of its pathology and the consequences of the seizures are thought to be restricted to cortex and thalamus (without effects in the limbic system). On the other hand, antiepileptogenesis has been found to reduce the cortical and cortico-thalamic network excitability and DTI changes in the absence network and beyond, suggesting that absence epilepsy has also consequences for the limbic system and that at least some of the consequences of epileptogenesis can be prevented.

Behavioral Studies Show a Comorbidity With Depression

Using these genetically absence epileptic rats, we have described their behavioral characteristics, their performance in learning and memory tests, as well as their coping with stressful events Earlier studies showed only minimal behavioral differences from controls in WAG/Rij rats, some disturbances in reference memory in WAG/Rij rats, and (surprisingly) improved avoidance learning in

GAERS. In experiments with graded novelty stress, WAG/Rij rats show a locomotor hyperresponse to mild environmental stress, but they react with remarkable passive behavior to stronger emotional stress (from which they cannot escape). Further behavioral tests by Sarkisova showed that WAG/Rij rats exhibit depressive-like features, such as increased immobility in Porsolt's forced swim test and decreased sucrose intake (anhedonia). Interestingly, WAG-Rij rats exhibit normalization of this behavior after chronic (but not acute) treatment with antidepressant drugs. Moreover, antiepileptogenesis by chronic and early drug treatment may reduce also the depressive-like phenotype. These findings validate the WAG/Rij strain as a genetic absence model with comorbidity for depression. In all, it is proposed that WAG/Rij rats are more vulnerable to stress than control rats and have depressive-like symptoms. Also in GAERS depressive and schizophrenic types of behavior were found.

Behavioral and neurochemical studies have also been done in order to investigate putative causes and mechanisms of the increased vulnerability to the depressive-like phenotype. A hyperresponse to amphetamine is found, similar to results in anhedonic depressive patients. The hyperresponse to amphetamine might be due to a high reactivity in the mesolimbic dopaminergic system. The sensitivity for the dopamine agonist, apomorphine, is also enhanced, suggesting that the dorsal striatum is involved in the phenotype. Also, indices of activity of the 5-HT system are found to be reduced in the forebrain of WAG/Rij rats, while the 5-HT response to inescapable sound stress is enhanced in the thalamus. Based on these observations, it has been proposed that absence epileptic rats are more vulnerable to stress in which the 5-HT system is involved, and that they represent a model for mild depression in which the dopaminergic system also seems to be involved. The outcome is clinically relevant, considering that both the absence seizures and the depressive symptoms need to be treated in depressed epileptic patients.

Electrical Stimulation Studies

In contrast to what is often believed, SWDs can be relatively easy (low intensity) interrupted by 1 s mono or biphasic high frequency stimulation at various points within the absence network, but also by stimulation in brain structures that modulate this network. In contrast, continuous high frequency stimulation (HFS, 130 Hz) of the sub-thalamic nucleus, which projects to the substantia nigra, a structure providing inhibitory control of convulsive and nonconvulsive generalized seizures, only temporarily (within the first 2 min) suppressed SWD, while thereafter habituation to stimulation occurred and SWDs reappeared. In contrast, various types of closed loop HFS of the same area yielded a 50% SWD interruption, analysis of the not successfully interrupted SWD revealed that those most often occurred shortly following a disrupted SWD, which might indicate the existence of a refractory period in which SWD disruption is less well possible. Furthermore, an increase in SWDs occurred. Stable SWD disruption was obtained outside this refractory period of ca 40 s of 97% during a 5 h stimulation (60 Hz) session and of 72% during a 24 h electrical stimulation session. A rebound was confirmed for the 5 h stimulation session, not for the 24 h stimulation session. Thalamic (VPM and ATN) HFS (130 Hz) closed loop stimulation for 8 h did not induce a rebound while 89% of SWD were interrupted at stimulation intensities (unilateral) as low as of 71.6 μA (VPM) and 113.6 μA (ATN), without changing the activity level of the animals. An automated closed loop seizure control system was used to evaluate the efficacy of an SWD interrupting system via stimulation (800 Hz, 0.5 s pulse train, pulse duration 0.5 ms, 30–40 μA) of the zona incerta. About 95% of the spontaneous SWDs and about 70% of the PTZ induced were terminated by a single train of electrical stimulation in a 4 h ECoG recording and stimulation session.

Although low frequency stimulation is commonly considered as proepileptic, SWDs were successfully interrupted by transcranial cortical stimulation (TES) with two or three strip of six bilateral electrodes either on the left of right parietal cortex and at the frontal midline, leading to a $>$60% diminution of both the mean duration and total duration of SWDs. This reduction was achieved by presenting a Gaussian waveforms of 50-ms TES after the detected spike with a timed delay of stimulus onset of 0, 10 or 40 ms so that TES coincided with the spike, or with the onset or the peak of the wave component of the SWD pattern. No clear differences were reported for the different delays, although it was suggested that widespread cortical stimulation "quenched the ongoing rhythm by recruiting subsets of thalamic cells, which in turn became refractory during the duty phase of the native SW cycle."

Future Challenges

Progress in science is guided by theories, and a new theory on the origin of the electroencephalographic absence seizures—the cortical focus theory for absence epilepsy—has been derived from work on genetic animal models of SWD epilepsy. An immediate challenge is to test this theory in humans, with the same and other signal analytical tools as used in rats. High resolution EEG and MEG have already demonstrated that there are cortical focal zones, which drive SWD. Also combined EEG and fMRI studies having the advantage of both temporal and spatial high resolution techniques are imperative, what has done so far seems in line with results in rats, as far as anatomical differences between rodent's and the human brain hamper a direct comparison. Such an comparison of the imaging results in rodents and patients is beyond the focus of this paper.

An unifying theory on the origin of epileptogenesis in the rodent models is still missing and it seems rather certain that absence epilepsy does not has a single cause. It is more likely that different processes all may lead to the appearance of SWD within the cortico-thalamo-cortical circuit. A theory in which the various findings—such as the upregulation of the various types of Ca^{2+} channels, the anomalies of I_h, glutamatergic and GABAergic neurotransmission in cortex and thalamus and the lack of some of the GABA-subunits in the thalamus including the RTN—are integrated, is needed. This theory should also include a least some of the mutated genes in absence epilepsy in humans, they have not been completely identified since variations in a couple of genes (ECA2 conferred by variation in the GABRG2 gene, ECA4 conferred by variation in the GABRA1 gene, ECA5 (GABRB3

gene) and ECA6 (CACNA1H gene) related to absence were not present in all CAE patients. There it is safe to conclude that there is a polygenetic genetic heterogeneity of susceptibility to childhood absence epilepsy. To the best of my knowledge, these genes were not investigated in the WAG/Rij and GAERS. Another putative interesting candidate gene is the gene that is responsible for the increased thalamic tonic inhibition in GAERS, since this increased tonic GABA-A receptor activation was not only observed in GAERS but also in other genetic mouse absence models such as stargazer and lethargic, but not in tottering mice. Increased tonic inhibition was due to compromised GABA uptake by the GABA transporter GAT1 (SLC6A1 gene) in the thalamus. Blockade or knockout of GAT1 in normal animals induced absence-like seizures and mice without thalamic GABA-A receptors were resistant to pharmacologically induced seizures. Therefore, the hunt for the genes controlling absence epilepsy is still open. Candidate genes might come from analysis of the genetic rat models. Micro-array technology may help to identify the expression levels of specific genes in the focal zone of the absence models; the specificity of the expression should be tested in the peri-oral zone of nonepileptic control rats. By including young, still not epileptic, WAG/Rij rats in such a study, and appropriate nonepileptic age matched control groups, it is possible to discriminate between genes that are affected by the disease rather than by the cause.

Molecular studies are helping to identify the effects of genetic deficits and its consequences, and to specify brain areas and their interconnections involved in the pathogenesis of epilepsy. The discovery of the subunits forming a large variety of GABA and glutamatergic ionophoric ion channel gated receptors with different distribution profiles, and the many subclasses of metabotropic glutamate receptors and neuromodulators, provide a broad panorama of means to target selectively and specifically the affected brain regions. Data from knockout mice are helpful and supplementary. To illustrate this point, it is worth noting that mice missing the mGlu4 receptor are completely resistant to the induction of absence seizures. These mGlu4 receptors are present presynaptically in cortico-thalamic neurons in the RTN and cortex. A positive allosteric modulator of this receptor, PHCCC, enhances dose-dependently, absence seizures, as shown by Richard Ngomba and coworkers. This finding suggests that the mGlu4 receptor antagonist might be a new tool for targeting SWDs. But also group I mGluR's: group I PAMS inhibit dose and time dependently SWDs in cortex and thalamus without inducing tolerance in a twice daily stimulation protocol, without clear adverse or behavioral effects. New and different antiabsence drugs are imperative considering that ethosuximide is not always effective or tolerated.

Besides reducing seizures by the development of new antiabsence drugs, treatment strategies that will slow or halt the progressive nature of epilepsy, and prevent the development of epilepsy in susceptible individuals, are needed. While it now well established that antiepileptogenesis is possible, its mechanisms needs to be established. Considering that different antiabsence and even a proabsence drug induces antiepileptogenesis in the genetic rat models, it is possible that signaling pathways might be involved. Other challenges are to develop methods for seizure prediction since precursor activity in the form of "hidden" network activity has been described in cortex and between cortex and thalamus. Other experimental therapies need to be explored, such as stem cell therapy and gene therapy. Considering that a focal region has been established, these therapies can be guided by this theory.

Contribution History

Since 2007 progress has been made regarding the cortical focus theory of absence epilepsy. The theory is now much more established, including confirmative evidence in patients. It is also accepted that absence epilepsy is considered and viewed as being a network type of epilepsy with a focal cortical onset. This is reflected in a large paragraph on the outcomes and new insights of different types of network approaches and the new **Figs. 4** and **5**.

It is now also more clear that, and although absence epilepsy is a multifactorial genetic disease, modification in the form of early treatment-manipulations and drug induced antiepileptogenesis with different drugs is feasible. A paragraph on antiepileptogenesis has been added. New is that brain imaging techniques also have shown disease modifying effects in the brain beyond the cortico-thalamo-cortical system. Also changes in excitability in the limbic system and in mood after antiepileptogenesis were added to this version.

Since 2007 new treatment and intervention options were explored, such as new drugs, and various types of electrical stimulation. It has become evident that SWDs can be easily aborted by various types of cortical and transcranial stimulation, but that long term studies regarding its therapeutic effects are necessary in order to get a better insight in their potential. A paragraph on the effects of various types of electrical stimulation on absence seizures has been added to the manuscript.

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